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Agriculture Development Fund

FINAL REPORT

INTRADERMAL VACCINATION: A MEANS TO ELIMINATE COSTLY SITE REACTIONS

19960084

Funded by
The Agriculture Development Fund

December 2000

Prepared by: University of Saskatchewan

SAF
Saskatchewan Agriculture & Food

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102-00038/

Project 9600084

Final Report to the Saskatchewan Agriculture Development Fund for the Research Project Titlted:

"Intradermal Vaccination: A Means to Eliminate Costly Site Reactions"

JA Ellis
Department of Veterinary Microbiology, Western College of Veterinary Medicine, University of Saskatchewan

Abstract / Summary

Vaccination site reactions cost producers millions of dollars each year due to condemnation. Disease reduction by vaccination is economically justifiable and widely practiced. Identification of alternative routes of administration that eliminate costly site reactions, would therefore be cost effective and provide a more consistent high quality product. The objective of this study was to examine intradermal vaccination as a means of effectively stimulating immune responses while eliminating costly injection site reactions. To date we have identified an injector that consistently, effectively, and painlessly delivers vaccine into the dermis of cattle. We have used this device to administer a fractionated doses (1/20 recommended dose) of combination vaccines to cattle. Most cattle that received intradermal vaccinations of 1/4 to 1/20 the recommended dose of commercial vaccines had antibody responses to BHV-1, BVDV and BRSV, as well as cell-mediated responses to BHV-1 and BRSV. Responses to bacterial pathogens were less consistent in both cattle and goats. These results indicate that intradermal delivery of vaccines, especially for viral pathogens may be an attractive to current delivery methods.

Executive Summary

Beef producers worldwide are concerned about identifying and eliminating factors that contribute to the variable quality and consistency in their product, since consumers cite inconsistency as a major reason why they are less likely to purchase beef (compared to poultry and pork) in restaurants and supermarkets. Recently the Canadian Beef Quality Audit outlined strategies to increase market share. One of these was to eliminate management practices that create nonconformity in beef and thereby increase consumer confidence in the product. They further cited intramuscular vaccination as 1 of the main practices to target for elimination.

Vaccination has been and continues to be the primary biological control used in the prevention of infectious disease in livestock. It is relatively inexpensive and without the potential residue concerns associated with the prophylactic and therapeutic use of pharmaceuticals. However, there has been increasing concern about injection site reactions in cattle. As recently documented in the Canadian Beef Quality Audit, injection site lesions cost Canadian cattlemen $26,317,272.00 annually, due to condemnation. Therefore, injection site reactions have been targeted for elimination. The purpose of this project is to examine intradermal vaccination as an alternate means of delivery of currently available vaccines. Intradermal vaccination could completely eliminate injection site reactions resulting from intramuscular (IM) and subcutaneous (SC) delivery of vaccines, and also result in a significant reduction in vaccine costs, since intradermal inoculation may require only a small fraction of the volume of vaccine used for conventional IM and SC routes of administration.

The results of this study, that used currently available delivery technology, demonstrated that small doses of vaccine can be delivered rapidly, consistently, efficiently, and painlessly into the dermis of cattle. Moreover, was shown that cattle

that receive fractionated doses (1/4 to 1/20) dose of commercially available combination vaccines develop significant increases in antibody and cellular immune responses to important viral pathogens including BHV-1, BRSV and BVDV. Cellular immune responses to BHV-1 were equivalent between groups of cattle that received 2 ml of vaccine compared to those that received 0.1 ml of vaccine. Antibody responses to viral pathogens were, however, significantly less (1/3-2/3) than in cattle that received 20 times more vaccine.

These results were encouraging and indicate that further examination of intradermal delivery is warranted. Even if the dose of vaccine required to achieve equivalent immune responses is not significantly reduced using intradermal vaccination, it is apparent that intradermal vaccination will completely eliminate costly site reactions in the muscle, and thereby result in considerable savings to the livestock producer.

Technical Report

I. EVALUATION OF INJECTION DEVICES

The Med-E-Jet injector gun and performed preliminary experiments in several cattle. In summary we found that the device could deliver intradermal injections in cattle, as reported in humans. However several problems were noted that would probably preclude its use in most livestock operations: 1) Lack of consistency; 2) There was some problem injecting through hair; studies with India ink indicated that there was often residual material on the hair. 3) The injector, that is driven by a CO2 cartridge is noisy and perhaps painful since cattle often jump upon injection. This could cause management problems in handling large numbers of cattle.

A spring-driven jet injector manufactured by DeAntonio Consultants International, Syracuse, New York, was also evaluated. In summary, comparative evaluation of DCI injector indicated that it is able to deliver small quantities of

vaccine into the dermis of cattle (and other species tested). It was quiet and painless; cattle were apparently unaware of an injection using this device.

Based on this comparative testing of both injectors, the DCI injector superior, however, further development of this or a similar device will be necessary. The models tested, while consistent, would require further modifications so that multiple doses of vaccines could more conveniently loaded and delivered to cattle under commercial handling conditions.

2) COMPARATIVE EVALUATION OF IMMUNE RESPONSES STIMULATED BY INTRADERMAL ADMINISTRATION OF VACCINES.

A. RESPONSES TO VIRAL ANTIGENS

Experiment 1. This was conducted using 48 Replacement heifers. Heifers were randomly assigned to 1 of 3 groups containing 16 cattle: Group 1 received 2-2 ml doses of a mixed vaccine containing modified-live BHV-1, BVDV, and PI3 in combination with a *Haemophilus somnus* bacterin administered intramuscularly as per label instructions; Group 2 received 0.1 ml of the same vaccine administered intradermally with the jet injector; Group 3 were commingled unvaccinated controls Cattle were vaccinated twice, three weeks apart and blood was collected 10 days after the second vaccination for assessment of antibody and cellular immune responses.

Cattle that received intradermal inoculation seroconverted to BHV-1 (GMT=47.1 + 4.7 ELISA units) but had significantly lower antibody responses than cattle that received 20x more vaccine intramuscularly (GMT=74.1 + 2.5 ELISA units). Similarly, cattle that received vaccine intradermally had group mean BVDV specific antibody titers (GMT=1/9) that were significantly higher than controls (GMT=<1/6), but significantly lower than cattle that received vaccine intramuscularly (GMT=1/103). Cellular immune responses as assessed by interferon

gamma release by cultured leukocytes (Figure 1) Most vaccinated cattle exhibited BHV-1 specific interferon gamma release and there were no significant differences between the cattle that received vaccine intramuscularly versus intradermally. Unvaccinated cattle did not seroconvert to BHV-1 or BVDV and had significantly lower BHV-1-specific interferon gamma release.

Experiment 2. This was conducted using 34 feedlot steers in a local commercial operation. Since this is a commercial operation all cattle were randomly assigned to 1 of 2 vaccinated groups: Group 1 received 2-2 ml doses of a mixed vaccine containing modified-live BHV-1, BRSV, and PI3 in combination with inactivated BVDV administered intramuscularly as per label instructions and a single dose of a combination 9-way clostridial vaccine (booster to primary immunization at branding) ; Group 2 received 0.1 ml of both the combination viral vaccine and the bacterin administered intradermally with the jet injector. Similar to the results obtained in experiment 1, most cattle that were vaccinated either intradermally or intramuscularly had significant increases in BHV-1 and BRSV-specific antibody titers after vaccination; in both cases the titers in the group that received a 1/20 dose intradermally (BHV-1, GMT=30.4 + 3.0 ELISA units; BRSV, GMT= 1/20) were significantly lower than herdmates that received 20 times more vaccine intramuscularly (BHV-1, GMT=52.6 + 3.2 ELISA units; BRSV, GMT= (1/58). Most steers in the group had high antibody titers to BVDV prior to vaccination and did not respond with increases in antibody titers after vaccination.

Experiment 3. In order to determine the effect of antigen dose and adjuvant on the induction of cellular and antibody responses, weanling beef calves twenty nine weanling beef calves were randomly assigned to 4 groups of 3 or 4 calves that received the following intradermal immunizations: Group 1, high dose killed BRSV; Group 2, low dose killed BRSV; Group 3, high dose killed BRSV and alum; Group 4, low dose killed BRSV and alum; Group 5 high dose modified live (MLV) BRSV;

Group 6, medium dose MLV BRSV Group 7, low dose MLV BRSV; Group 8, low dose MLV BRSV and alum; Group 9, tissue culture (control). Vaccines were given in 0.8-1.0 ml doses intradermally in the cervical region. The low dose MLV vaccine skewed the response towards a cellular (T helper 1?) response while the high dose killed vaccines skewed the responses toward an antibody response (T helper 2?) (Figures 2-4).

Experiment 4. Newborn calves were deprived of maternal colostrum and fed 2 liters of either BVDV or BRSV- antibody negative colostrum, in a divided dose in the first 18 hour of life. Groups of Four to six week old calves were inoculated intramuscularly with a mixed vaccine containing modified-live BVDV and BRSV; 6 calves received 0.25 cc of the vacccine intradermally in 2 sites, one on each side of the neck, 6 calves received 2 ml IM and 6 calves were unvaccinated controls. Calves receiving the BRSV vaccine were vaccinated twice, two weeks apart and tested 2 weeks later. Calves receiving the BVDV vaccine were vaccinated once and tested approximately 150 days later. Intramuscular and intradermal delivery of the vaccine stimulated equivalent responses (Figures 5-8)

B. RESPONSES TO BACTERIAL ANTIGENS

Experiment 1. This was conducted in 46 goats in herd of goats used for antibody production at Santa Cruz Biotechnology that has a large herd that is routinely vaccinated with a commercial mixed vaccine containing *Corynebacterium pseudotuberculosis*. Goats were vaccinated by 2 different routes. Twenty-three goats were inoculated twice with 1 ml of vaccine in the subcutis according to manufacture's recommendations; 23 herdmates were inoculated twice with 0.1 ml of vaccine intradermally (delivered with a tuberculin syringe). Most of the goats that were vaccinated intradermally had significant increases *C. pseudotuberculosis*-specific antibodies (GMT=21.4 ELISA units); however, antibody titers were

significantly lower than goats that received 10x more vaccine in the subcutis (GMT=75 ELISA units). Interestingly, the cellular responses, as indicated specific interferon gamma release were higher in the goats that received vaccine intradermally, although there were no significant differences between groups.

Experiment 2. Sera collected from cattle in Experiment A.1. were assayed for isotype antibody responses *to H. Somnus* in collaboration with Dr. L. Corbeil, University of California, San Diego. Both IM and ID vaccines stimulated 5-6 times increase in *H.Somnus* specific IgG1 and IgG2 and there were no apparent differences between groups or isotypes. However, unvaccinated control cattle also seroconverted to *H. Somnus*. This occurred despite isolation of the cattle from other animals and suggested exposure prior to or at about the time of vaccination. These results are therefore difficult to interpret.

Experiment 3. Sera collected from cattle in Experiment A.2. were assayed for by ELISA for antibody responses to Clostridial antigens (Clostridial vaccine used as antigen. Cattle the were vaccinated by both the intramuscular and intradermal route had significantly increased antibodies after vaccination; however, the cattle that were vaccinated with the higher dose intramuscularly had significantly higher titers (Figure 9)

Discussion

The results of this study were encouraging for future development of intradermal vaccines for use in cattle and other species. Intradermal administration of vaccines may not only further reduce the prevalence of site reactions and stress and trauma associated with routine processing, but is also potentially more cost effective. Based on the results of this study and subsequent challenge experiments funded by other agencies, the following conclusions can be drawn:

1) With current delivery technology vaccine can be administered, rapidly, consistently, and painlessly into the dermis of cattle. However, the current technology for intradermal vaccination is still not totally "user friendly". Further development is required so that multiple doses of vaccines can be more efficiently loaded and delivered more rapidly, so that intradermal delivery could be implemented in commercial livestock operations.

2) Use of 1/20 the dose of commercially available vaccines stimulated significant increases in antibody responses and cellular responses to viral pathogens; albeit antibody responses were consistently significantly lower in cattle that receive vaccine at 1/20 the recommended dose. Use of 1/4 of the total recommended dose of combination viral vaccines administered in 2 intradermal sites stimulated immune responses that were not significantly different from those obatined with conventional delivery of 2 ml of vaccine the same vaccine. More importantly, in studies conducted as a followup to this project, young cattle that were immunized intradermally with 1/4 the recommended dose of vaccine developed protective immunity to BVDV or BRSV challenge that was not signifantly different from that in calves that received the recommended dose of vaccine delivered via the conventional intramuscular route.

3) Intradermal delivery of fractionated doses of representative bacterial antigens *C. pseudotuberculosis* and *H. somnus* resulted in antibody responses that were significantly less than those resulting from conventional delivery of the full dose of vaccine. This could be due to the a requirement for higher antigenic mass for bacterial antigens. These responses could possibly be enchanced with inclusion of different adjuvants, which would require further development. Nevertheless, challenge experiments are required to determine if the immune responses induced by intradermal

delivery, while quantatively reduced, may be sufficient to prime the animal for protective anamnestic response upon subsequent exposure to the pathogen. Further investigation of alternative (intradermal) delivery systems for common bacterial vaccines is warranted for at least 2 reasons: 1) that bacterial antigens or bacterins are among the most commonly used immunogens, and 2) bacterial antigens, as perhaps best exemplified by clostridial vaccines, tend to be particularly irritating, and are often given in large volume (>2 ml).

In conclusion the results of this study indicate that intradermal delivery of comparatively small doses of both viral, and to a lesser extent, bacterial antigens may be an attractive alternative to current vaccination practices. Even if intradermal delivery is unable to achieve savings in terms of reduced vaccine costs (i.e. reduction in vaccine volume / dose required) , it will almost completely costly site reactions, as well as considerably reduce the stress and resultant negative economic impact of traditional "needle-driven" processing procedures.

Project Activities, Achievements, Papers, Presentations

These data have been presented or referred to in several intramural and extramural seminars, including the annual meeting for both practicing veterinarians and producers. These forums were efficient means of transmission of these findings; however, since the doses of commercial vaccines used were "off label",

Indications for Further Technical or Commerical Development

These data indicate the clear potential for further commercial development of intradermal vaccines and intradermal delivery systems, especially for viral vaccines

Information of Benefit to Producer's, Government or Industry

These studies entailing evaluation of available technology for intradermal vaccine delivery indicated that intradermal delivery of vaccines is an attractive alternative to

traditional vaccination procedures that could result in the elimination of costly injection site reactions. Commercial availability of vaccines and delivery systems for intradermal vaccines will require further development by industry.

Personnel

Carrie Konoby (Technician level II) was employed throughout the duration of the project. Dr. Keith West, an Interprovincial Scholar obtained his PhD.in the area of vaccine-stimialted immunity to BRSV. An evaluation of intradermal delivery of commerically available BRSV vaccines was part of that work.

Eqiupment

Med-E-Jet injector

Project Developed Materials

None produced or anticipated.

Project Photos

There are photographs of of vaccine delivery which can be made available upon request.

SADF Acknowledgement

The support of SADF has been acknowledged in several seminars related at which these data were discussed.

Expense Statement

The statement of expenditures has been forwarded directly to the SADF offices from the University of Saskatchewan Office of Research Services.

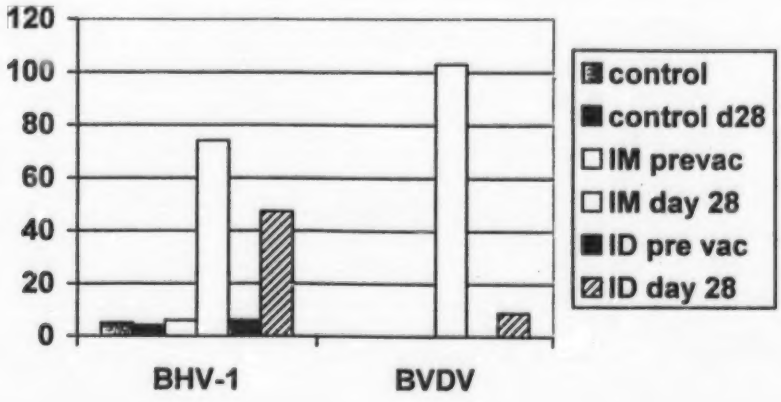

Figure 1. Group mean BHV 1 (assessed by ELISA) and BVDV-specific (assessed by virus neutralization) antibody before and after 2 doses of ID or IM immunization with total dose of 0.1 or 2.0 ml, ml respectively at each immuniztion (0 and 21 days). Values are ELISA units and reciprocal of geometric mean titers, respectively. Both the IM and ID group are significantlly different than unvaccinated controls; the IM group is significantly higher than the ID group in both cases.

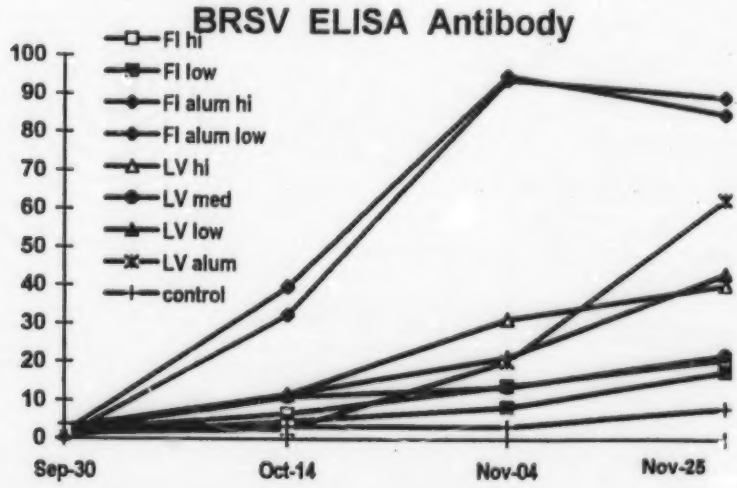

Figure 2. Group mean BRSV-specific IgG (as assessed by ELISA) after 2 ID doses (0 and 14 days). Values are ELISA units.

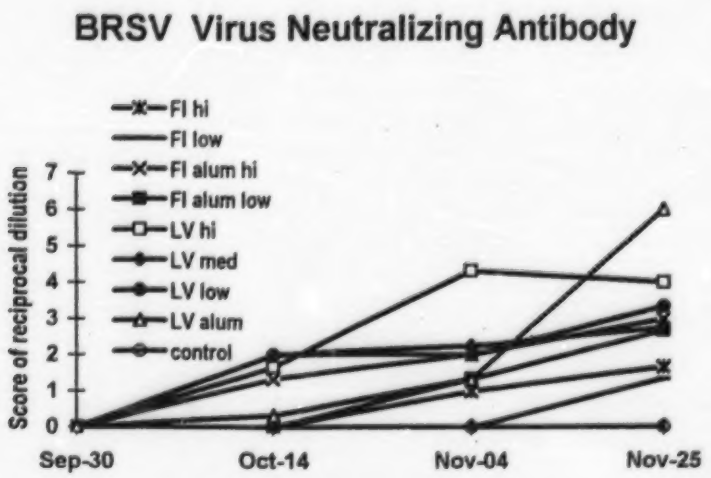

Figure 3. Group mean BRSV-specific neutralizing antibodies after 2 ID doses (0 and 14 days). Values are geometric mean titers.

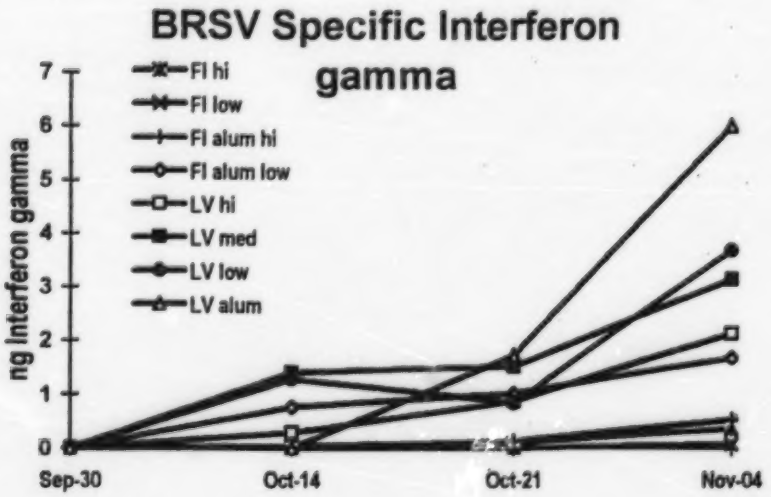

Figure 4. Group mean BRSV-specific interferon gamma after 2 ID doses (0 and 14 days). Values are nanograms of interferon gamma (in culture supernatant).

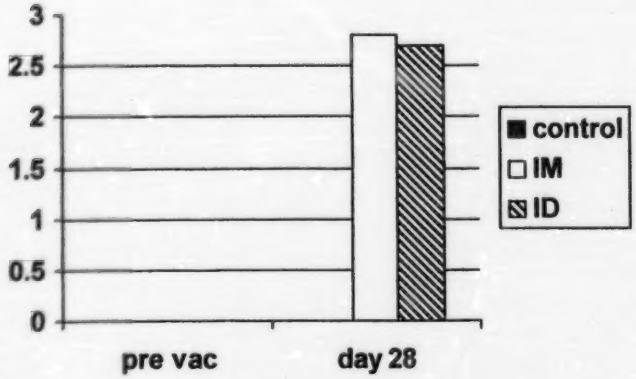

Figure 5. Group mean BRSV-specific neutralizing antibody before and after 2 doses of ID or IM, with total dose of 0.5 or 2.0ml, respectively, at each immuniztion (0 and 14 days). Values are geometric mean titers. Both the IM and ID group are significantly greater than unvaccinated controls.

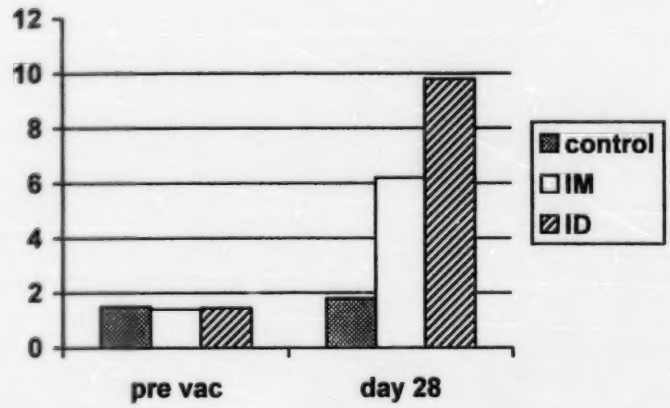

Figure 6. Group mean BRSV-specific lymphocyte blastogenesis before and after 2 doses of ID or IM, with total dose of 0.5 ml or 2.0 ml, respectively, at each immunization (0 and 14 days). Values are stimulation indicies. Both the IM and ID group are significantlly greater than unvaccinated controls and not significantly different from each other.

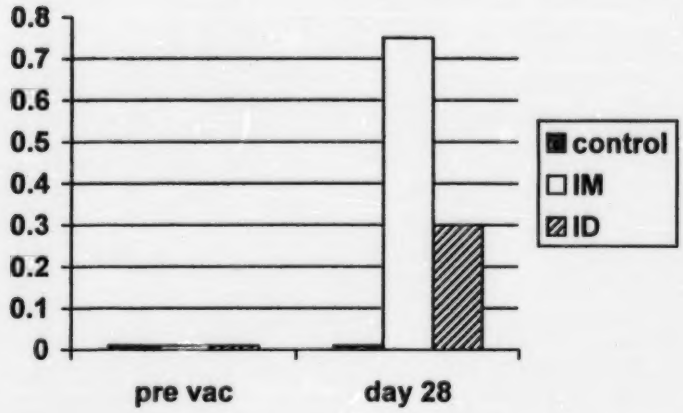

Figure 7. Group mean BRSV-specific interferon gamma before and after 2 doses of ID or IM, with total dose of 0.5 or 2.0 ml, respectively, at each immuniztion (0 and 14 days). Values are in nannograms of INF gamma (in leukocyte culture supernatant). Both the IM and ID group are significantlly greater than unvaccinated controls and not significantly different from each other.

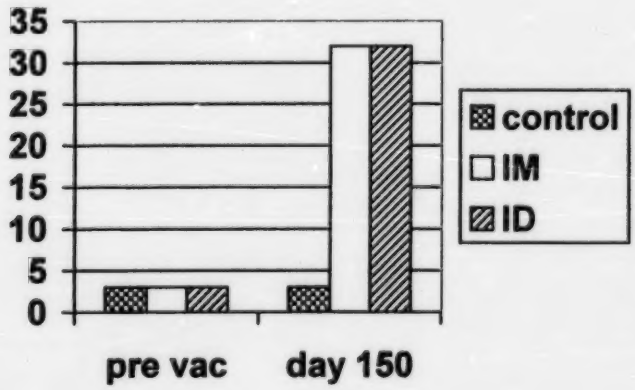

Figure 8. Group mean BVDV (type 1)-specific neutralizing antibody before and approximately 150 days after 1 ID or IM, with total dose of 0.5 or 2.0ml, respectively. Values are geometric mean titers. Both the IM and ID group are significantlly greater than unvaccinated controls.

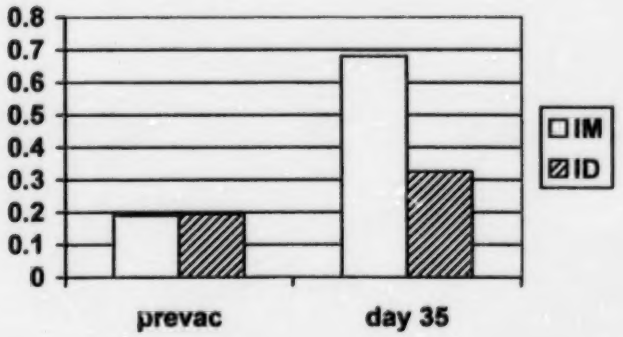

Figure 9. Group mean clostridial antigen-specific antibody (assessed by ELISA) before and after 1 dose of ID or IM 0.2 or 1.0 ml, ml respectively. Values are ELISA units. Both the IM and ID groups had significant increases in antibody concentrations after vaccination; however, the IM group mean was significantly higher than the ID group.